

Data acquisition of resting-state fMRI and optogenetic fMRI experiments

JL Jiaming Liu EW Ed X. Wu YF Yanqiu Feng XC Xiong Cao

Updated date: May 23, 2023

An abbreviated version of this protocol was published in Science Advances in Nov 2022

Astrocyte dysfunction drives abnormal resting-state functional connectivity in depression

DOI: 10.1126/sciadv.abo2098

Detailed protocol

Data acquisition protocol for resting-state fMRI and optogenetic fMRI experiments in mice

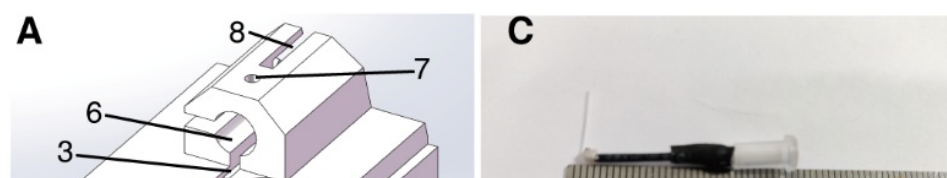
Setup before fMRI data acquisition

1. The mice were anesthetized with 3.5% isoflurane for 5 minutes.
2. Then, the mice were injected with a bolus of dexmedetomidine (0.02 mg/kg) intraperitoneally.
3. One drop of 2.5% lidocaine was then applied to the chords of mice to provide local anesthesia before endotracheal intubation.
4. The mice were positioned on an MRI-compatible cradle, fixed by ear bars and a bite bar, and connected to a ventilator (TOPO, Kent Scientific, USA) using a homemade 3D-printed mouse breathing mask (**Fig. 1, A and B**), which was compatible with mechanical ventilation under the Bruker mouse head crycoil (MRI CryoProbe, Bruker BioSpin, Germany). The mice were mechanically ventilated at a rate of 80 breaths per minute, with a respiration cycle of 25% inhalation and 75% exhalation (the gas source is medical oxygen).
5. The mice were connected to a variety of physiological detectors, including ones that measured respiration, rectal temperature, and oxygen saturation. Mouse rectal temperature was maintained at $37 \pm 0.1^\circ\text{C}$ with an MR-compatible heater system (Heater system, Small Animal Instruments, USA). Continuous physiological monitoring was performed throughout the duration of the experiments.
6. After the above setup, isoflurane was reduced and maintained at 0.4%, and a continuous subcutaneous infusion of dexmedetomidine (0.04 mg/kg per hour) was started to maintain the sedation level.
7. The above setup of mice took approximately 15 minutes. During this period, the physiological states of mice gradually stabilized to normal ranges (rectal temperature: $37 \pm 0.1^\circ\text{C}$, breathing: 80 breaths per minute, heart rate: 350 to 420 beats per minute, oxygen saturation: >95%) before the start of the fMRI data acquisition.

fMRI data acquisition

1. All fMRI experiments were performed on a Bruker 7T MRI scanner (Bruker BioSpin, Germany) equipped with a mouse head crycoil.
2. Data acquisition includes localizer scanning, high-resolution anatomical image acquisition, B0 map acquisition, and fMRI image acquisition.
 - **Turbo-RARE:** The anatomical images were acquired using a spin echo (Turbo-RARE) sequence [field of view (FOV) = $16 \times 16 \text{ mm}^2$, matrix = 256×256 , RARE factor = 8, repetition time (TR)/echo time (TE) = 2500/35 ms, slice thickness = 0.4 mm].
 - **GE-EPI:** Local field homogeneity was optimized in the brain using the B0 field maps scanned in advance. fMRI images were then obtained using a single-shot gradient echo planar imaging (GE-EPI) sequence with FOV = $16 \times 16 \text{ mm}^2$, matrix = 64×64 , flip angle = 54.7° , slice thickness = 0.4 mm, dummy scans = 10, TE = 15 ms, TR = 750 ms (resting-state fMRI) or 1000 ms (optogenetic fMRI), averages = 1, Segments = 1, Slice gap = 0, repetitions = 450 (resting-state fMRI) or 360 (optogenetic fMRI). fMRI image acquisition has the same slice geometry imported from the previously acquired T2-weighted anatomical image.
 - **An Arduino programming board was used to synchronize the scanner trigger and the optogenetic stimulation laser for optogenetic experiments.** A 473 nm blue light was delivered using a laser transmitter via an optical patch cable. The optogenetic stimulation (10 Hz; light power: 3.5 mW at the fiber tip; 15% duty cycle) was delivered with a duration of 20 s.

Image



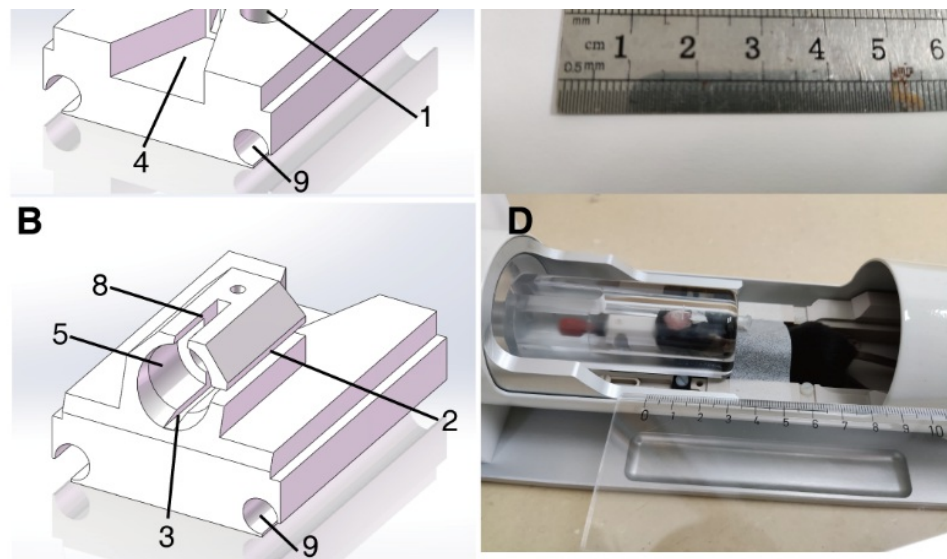


Fig. 1. Homemade 3D-printed mouse breathing mask compatible with endotracheal intubation under the Bruker mouse head crycoil (A-B), and right-angle optical fiber (C-D). A-B, Mouse fixed mask compatible with endotracheal intubation. Use the steps description details of fixed mask: (1) After endotracheal intubation of mice, one end of the ventilation tube was connected to the trachea of mice, and the other end was connected to the ventilation through the mask extension tube 9. The inlet and outlet ventilation tube are connected to the ventilator via the left and right mask extension ducts 9 respectively. (2) Place the cannula tube across the mask side slot 2 and down into slot 3. (3) The mouse bite bar is inserted through cavity 6, hooked onto the incisors of the mouse, pulled into cavity 5, and fixed by turning the screw at thread 7. (4) The junction between the cannula tube and the ventilation tube was placed in the trigeminal slot 4 and secure it with medical tape. (5) Screw the screw at thread 1 to fix the fixed mask to the Bruker imaging animal bed. (6) Observe the position of the mouse through window 8, and adjust the position of the bite bar if it is not in the central position. (7) Fixing of mice using Bruker's ear bars. **C,** Right-angle optical fiber. **D,** A representation mouse implanted with right-angle optical fiber.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Liu, J. , Wu, E. , Feng, Y. and Cao, X. (2023). Data acquisition of resting-state fMRI and optogenetic fMRI experiments. Bio-protocol Preprint. [bio-protocol.org/prep2312](https://doi.org/10.2196/preprint2312).
2. Liu, J., Mo, J., Wang, X., An, Z., Zhang, S., Zhang, C., Yi, P., Leong, A. T. L., Ren, J., Chen, L., Mo, R., Xie, Y., Feng, Q., Chen, W., Gao, T., Wu, E. X., Feng, Y. and Cao, X.(2022). Astrocyte dysfunction drives abnormal resting-state functional connectivity in depression. Science Advances 8(46). DOI: [10.1126/sciadv.abo2098](https://doi.org/10.1126/sciadv.abo2098)

Copyright: © 2023 The Author(s); This is an open-access article under the CC BY-NC license (<https://creativecommons.org/licenses/by-nc/4.0/>).